Population structure analyses of Staphylococcus aureus at Tygerberg Hospital, South Africa, reveals a diverse population, a high prevalence of Panton-Valentine leukocidin genes, and unique local methicillin-resistant S. aureus clones

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Population structure analyses of *Staphylococcus aureus* at Tygerberg Hospital, South Africa, reveals a diverse population, a high prevalence of Panton–Valentine leukocidin genes, and unique local methicillin-resistant *S. aureus* clones

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Abstract

Studies reporting on the population structure of *Staphylococcus aureus* in South Africa have focused only on methicillin-resistant *S. aureus* (MRSA). This study describes the population structure of *S. aureus*, including methicillin-susceptible *S. aureus* (MSSA) isolated from patients at Tygerberg Academic Hospital, Western Cape province. Pulsed-field gel electrophoresis (PFGE), detection of Panton–Valentine leukocidin (PVL), *spa* typing, multilocus sequence typing (MLST), *agr* typing and SCC*mec* typing were used to characterize strains. Of 367 non-repetitive *S. aureus* isolates collected over a period of 1 year, 56 (15.3%) were MRSA. Skin and soft tissue infections were the most frequent source (54.8%), followed by bone and joint (15.3%) and respiratory tract infections (7.7%). For strain typing, PFGE was the most discriminative method, and resulted in 31 pulsotypes (n = 345, 94.0%), as compared with 16 *spa* clonal complexes (CCs) (n = 344, 93.4%). Four MLST CCs were identified after eBURST of sequence types (STs) of selected isolates. One hundred and sixty isolates (MSSA, n = 155, 42.2%) were PVL-positive, and *agr* types I–IV and SCC*mec* types I–V were identified. Our *S. aureus* population consisted of genotypically diverse strains, with PVL being a common characteristic of MSSA. MSSA and MRSA isolates clustered in different clones. However, the dominant MRSA clone (ST612) also contained an MSSA isolate, and had a unique genotype. Common global epidemic MRSA clones, such as ST239-MRSA-III and ST36-MRSA-II, were identified. A local clone, ST612-MRSA-IV, was found to be the dominant MRSA clone.

Keywords: Methicillin resistance, PVL, *S. aureus*, SCC*mec*, South Africa
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Introduction

Staphylococcus aureus is a facultative intracellular bacterium that is considered to be one of the most important and significant human pathogens. Methicillin-resistant *S. aureus* (MRSA) emerged as a major clinical and epidemiological hospital-associated problem in the 1980s [1], even though Jevons described the first MRSA isolate in the 1960s [2]. SCC*mec* was first described as a novel mobile genomic element, capable of site-specific and orientation-specific integration into and precise excision from any *S. aureus* strain [3].

Typing techniques are required for the effective discrimination and characterization of isolates in order to establish clonal relatedness and clonal dominance. Numerous molecular techniques have been described for *S. aureus* typing, of which macrorestriction analysis (MRA) with pulsed-field gel electrophoresis (PFGE) is still regarded as the reference standard, as it is still the most discriminatory technique available for *S. aureus* [4].

Information regarding the molecular epidemiology and pathogenicity of *S. aureus* in South Africa is limited. The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) study indicated methicillin resistance to be 33.3% for South African strains isolated during 2001–2002 [5]. There is also a lack of data regarding community-associated MRSA (CA-MRSA) in Africa. A study on MRSA isolates collected nationally revealed the presence of the following internationally described MRSA clones: sequence type (ST) 5-MRSA-I, ST239-MRSA-III, ST612-MRSA-IV, ST36-MRSA-II, and ST22-MRSA-IV [6].

A genotypic study conducted at Tygerberg hospital on 113 *S. aureus* blood culture isolates by *spa* typing identified 49 different *spa* types that grouped into seven clusters [7]. *spa* type t037, associated with the Brazilian/Hungarian epidemic MRSA clone, was identified as the dominant *spa* type. ST612 was identified as a dominant MRSA clone in another study from Cape Town [8].

The aim of this study was to elucidate the population structure of *S. aureus* strains isolated from specific clinical sites of infection from patients admitted to Tygerberg hospital.

Materials and Methods

Study design and setting

This cross-sectional study was undertaken in the molecular laboratory of the Division of Medical Microbiology of the Faculty of Medicine and Health Sciences, Stellenbosch University, located at Tygerberg Hospital. This is a 1200-bed academic hospital in the Western Cape that serves a diverse population of approximately 2.5 million people, including areas with high human immunodeficiency virus (HIV) and tuberculosis prevalence rates. Annually, 60 600 patients are admitted, and the *S. aureus* prevalence rate is 3.2%.

Sample collection and patient demographics

Consecutive, non-duplicate, single-patient *S. aureus* isolates were collected over a 1-year period (2009–2010). Bacterial isolates were identified as *S. aureus* with the Vitek 2 system (Vitek 2 GP identification cards) (bioMérieux, Marcy l'Etoile, France), which was also used for antimicrobial susceptibility testing (Vitek 2 AST-P603 card). The isolates were subcultured on blood agar plates and stored at −80°C. Specimen type, infection source and methicillin resistance were recorded where available, as well as patient age, gender, and HIV status (if available). Isolates were classified as paediatric (≤12 years) or adult (≥13 years). The following isolates were obtained from the American Type Culture Collection (ATCC) (www.atcc.org) as control isolates: ATCC-BAA-38; ATCC-BAA-39; ATCC-BAA-42: ATCC-1680; ATCC-BAA-81; ATCC-BAA-83; ATCC-BAA-88; and ATCC-BAA-1707. Strain NCTC8325-4 was obtained from the Health Protection Agency of the UK (www.hpa.org.uk).

Genomic DNA extraction

Total genomic DNA was extracted with a previously published method [9].

Strain typing

PFGE. A previously published protocol using *Sma*I was used to type all isolates [10]. The banding patterns were used to establish isolate relatedness according to the criteria of Tenover *et al.* [11]. For PFGE pulsotypes where *Sma*I restriction digestion was unable to differentiate between the *spa* typing and multilocus sequence typing (MLST) clonal complex (CC) results within a particular PFGE pulsotype, *Apa*I macrorestriction was applied [12]. PFGE pulsotypes were classified on the basis of the number of isolates and unique PFGE types as major pulsotypes (more than ten isolates/PFGE types), intermediate pulsotypes (four to nine isolates/PFGE types), or minor pulsotypes (two or three isolates/PFGE types).

spa typing and MLST. *spa* typing was performed as previously described [13]. *spa* types were clustered into *spa*-CCs with the algorithm Based Upon Repeat Pattern (BURP) (RIDOM StaphType v.2.1.1; RIDOM, Würzburg, Germany) with a distance cost of ≤5; *spa* types shorter than four repeats were excluded [14]. MLST was performed for all PFGE pulsotypes classified as major or intermediate, as published previously [15]. Allelic profiles were obtained from the website http://saureus.mlst.net, and STs were clustered into CCs with eBURST v.3 (http://saureus.mlst.net/eburst).

SCCmec, *agr* and *lukS/F* detection. SCC*mec* typing was performed as described by Milheirico *et al.* [16]. The *agr* type of each isolate was identified as described by Lina *et al.* [17]. The *lukS/F* genes were detected according to a previously published protocol [18].

Statistical analyses

Association between clonality and clinical/bacterial characteristics. Fisher's exact test was used to determine associations between clonality and the following clinical/bacterial characteristics: (i) methicillin resistance; (ii) paediatric/adult clones; (iii) infection source; (iv) gender; and (v) Panton–Valentine leukocidin (PVL) status. p-Values were corrected for multiple analyses with the Holm–Bonferroni method. Fisher's exact test was also used to investigate the association between

methicillin resistance and *agr* type and PVL status. A significance level of 0.05 was used.

Discriminatory power of typing techniques. A discriminatory index with 95% CIs was calculated for PFGE and *spa* typing as published previously [19].

Results

Sample collection and methicillin resistance

Three hundred and sixty-seven *S. aureus* isolates were collected, of which 56 (15.3%) were MRSA.

Patient demographics and clinical sources of infection

One hundred and six isolates (28.9%) were classified as paediatric isolates, and 261 isolates (71.1%) were classified as adult isolates. Two hundred and eighteen isolates (59.4%) were collected from males, 148 isolates (40.3%) from females, and one (0.3%) from a post-mortem sample of unknown gender. The median age of patients was 30 years, and ranged from 3 days to 87 years. Eighty-five isolates (23.2%) were collected from persons with known HIV status, of which 21 (24.7%) were from HIV-positive persons, representing 5.7% of the whole collection. Three hundred and fifty-two isolates (95.9%) were collected from known clinical sources (Fig. 1).

Typing results

*Sma*I PFGE. Three hundred and sixty-six isolates (99.7%) were typeable by *Sma*I restriction analysis; this resulted in the generation of 269 unique PFGE types, grouped into 31 PFGE pulsotypes and 21 singletons at an 80% similarity cut-off. Two hundred and fifty-four (69.4%) of the typeable isolates grouped into eight major pulsotypes, 62 (16.9%) grouped into ten intermediate pulsotypes, and 29 (7.9%) grouped into 13 minor pulsotypes (Table 1). The pulsotypes identified were given customized names to facilitate analyses.

*Apa*I PFGE. *Apa*I MRA was performed on the pulsotype THW-V, as three *spa*-CCs and three *agr* variants were observed. MRA with *Apa*I clearly showed the presence of three clusters with an 80% cut-off, which correlated with the *agr* typing and *spa* clustering. A numerical index combined with the previously assigned alphabetical name from *Sma*I MRA was assigned, resulting in THW-V1, THW-V2, and THW-V3.

spa typing. The *spa* types of 366 isolates (99.7%) were determined. One hundred and twenty-seven unique *spa* types were identified. Thirty-five of these *spa* types were identified as novel types. In this study, 78 *spa* types (21.3%) represented single isolates. The dominant *spa* type was t891 ($n = 51$, 13.9%). With the BURP algorithm, the 127 *spa* types were clustered into 16 *spa*-CCs. The *spa* types of 14 isolates (3.8%) were classified as singletons, and eight *spa* types from seven isolates (1.9%) were excluded from the analyses because they were too short. *Spa*-CC891 was identified as the dominant *spa*-CC, comprising 64 isolates (17.5%) (Table 1).

SCCmec typing. SCC*mec* type IV was identified as the dominant variant, being present in 30 isolates (53.6%). SCC*mec* type I was identified in nine (16.1%) isolates, SCC*mec* type II in six (10.7%) isolates, and SCC*mec* types III and V in five (8.9%) isolates each. One isolate was non-typeable and yielded no amplification pattern, except for the *mecA* internal control.

agr typing. The *agr* types of all isolates were determined, and all four *agr* types were identified; *agr* type I was the dominant type, being identified in 199 isolates (54.2%). *agr* type III was identified in 80 isolates (21.8%), *agr* type II in 68 isolates

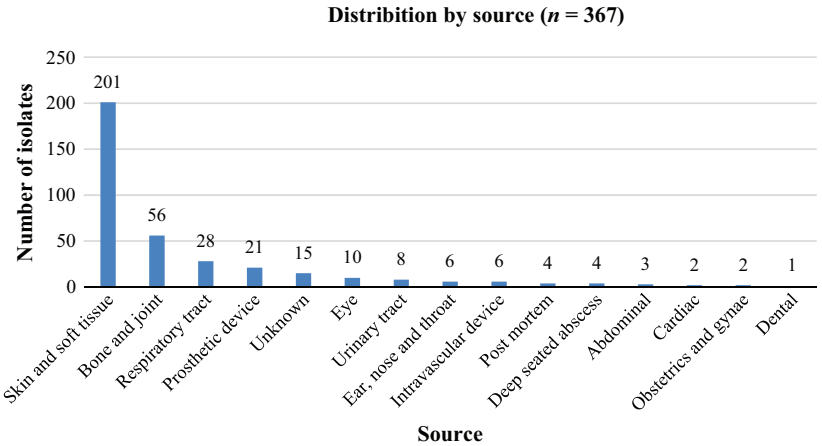

FIG. 1. The distribution of isolates collected according to source. The isolates are displayed as collected according to the clinical source, starting with the most prevalent source (skin and soft tissue). The number of isolates collected from each source is also indicated.

TABLE 1. Pulsed-field gel electrophoresis (PFGE) pulsotypes identified, with supporting data from other typing techniques

PFGE[a]	No. of isolates	Status of pulsotypes	MLST		*spa*-CC	SCC*mec* (types: number)	*agr*	No. PVL-positive (%)
			ST	CC[b]				
THW-A	46	Major	1865	30	21	MSSA	III	38 (83)
THW-D	3	Minor	–	–	NF	MSSA	I	0
THW-E	16	Major	121	121	NF	MSSA	IV	15 (94)
THW-F	3	Minor	–	–	NF	MSSA	IV	3 (100)
THW-G	7	Intermediate	1862	8	1597	MSSA	I	3 (43)
THW-H	2	Minor	–	–	64	MSSA	I	1 (50)
THW-K	2	Minor	–	–	64	MSSA	I	1 (50)
THW-M	2	Minor	–	–	64	MSSA	I	0
THW-Q	2	Minor	–	–	267	MSSA	III	2 (100)
THW-R	2	Minor	–	–	84	MSSA	II	0
THW-S	16	Major	15	15	84	MSSA	II	2 (13)
THW-T	2	Minor	–	–	84	MSSA	II	0
THW-U	2	Minor	–	–	191/100	MSSA	II	1 (50)
THW-V	6 (V3)[c]	Intermediate	12	12	888	MSSA	II	1 (17)
	14 (V2)[c]	Major	1	15	NF		III	5 (36)
	18 (V1)[c]	Major	6	6	64		I	1 (6)
	2	Minor	ND	ND	NF		III	2 (100)
THW-W	5	Intermediate	97	97	267	MSSA	I	0
THW-X	5	Intermediate	188	15	189	MSSA	I	1 (25)
THW-Y	17	Major	1863	5	2	MSSA	II	1 (6)
THW-Z	6	Intermediate	1864	5	2	MSSA	II	1 (17)
THW-CC	2	Minor	–	–	1597	MSSA	I	0
THW-DD	2	Minor	–	–	st	MSSA	I	2 (100)
THW-EE	37	Major	45	45	15	MSSA	I	1 (19)
THW-B	6	Intermediate	36	30	21	Type II: 6	III	0
THW-N	8	Intermediate	612	8	64	Type IV: 8	I	0
THW-P	2	Minor	–	–	64	Type IV: 2	I	0
THW-AA	9	Intermediate	5	5	2	Type I: 9	II	0
THW-L	6	Intermediate	239	8	21	Type III: 5 NT: 1	I	0
THW-C	63	Major	22	22	891	MSSA: 58	I	57 (98)
						Type V: 1		1 (100)
						Type IV: 4		0
THW-I	3	Minor	–	–	148	MSSA: 2	I	0
						Type V: 1		0
THW-J	6	Intermediate	8	8	64	MSSA: 4	I	1 (25)
						Type V: 2		0
THW-O	13	Major	612	8	64	MSSA: 1	I	0
						Type IV: 12		1 (8)
THW-BB	4	Intermediate	88	88	186	MSSA: 2	III	1 (50)
						Type IV: 2		2 (100)

CC, clonal complex; MLST, multilocus sequence typing; MSSA, methicillin-susceptible *Staphylococcus aureus*; NF, no founder; ND, not determined; NT, not typeable; st, singleton; ST, sequence type; +, positive.
[a]Isolates identified as singletons (n = 21) and non-typeable (n = 1) are not included in the table.
[b]CC8 was discussed as an individual CC for ease of interpretation and comparison with previously published literature, although recent data on the MLST website indicate that this CC is now a part of CC5.
[c]*Apa*I macrorestriction analysis.

(18.5%), and *agr* type IV in 20 isolates (5.4%). No association was identified between methicillin resistance and *agr* type.

PVL. One hundred and sixty isolates (43.6%) tested positive for *lukS/F*, of which 155 (42.2%) were methicillin-susceptible *S. aureus* (MSSA) isolates and five (1.4%) were MRSA isolates. MRSA isolates were associated with PVL negativity (p 0.0001).

MLST. A representative isolate was randomly selected for each major and intermediate PFGE pulsotype. Nineteen MLST STs were identified from 20 isolates, which clustered into two MLST CCs and 14 singletons, according to a similarity of six of seven loci. To group singletons into CCs, eBURST analysis was performed with all previous STs submitted to the database. This allowed us to group the STs into four CCs (CC30, CC8, CC15, and CC5) and seven singletons (ST22, ST121, ST45, ST6, ST12, ST97, and ST88) (Table 1). In this article, CC8 is referred to as separate from CC5 for ease of interpretation and comparison with other research articles, although CC8 has now been incorporated into CC5 (http://saureus.mlst.net/eburst).

Combined molecular typing data. A good correlation was seen after combination of the data obtained with the various typing techniques. All major (n = 9) and intermediate (n = 11) PFGE pulsotypes each had a unique *agr* type and *spa*-CC, and were predominantly or completely PVL-positive or PVL-negative. Only four PFGE pulsotypes consisted of both methicillin-susceptible and methicillin-resistant isolates: THW-C, THW-J, THW-O, and THW-BB. Each PFGE pulsotype had a unique ST, except for ST612, which was identified in both PFGE pulsotype THW-N and PFGE pulsotype THW-O. *Spa*-CCs were divided into numerous clones by both PFGE and MLST. A unique SCC*mec* type was present in all MRSA isolates from the same

PFGE pulsotype, except for THW-L where one isolate was non-typeable, and THW-C, where both SCC*mec* type IV (PVL-negative) and SCC*mec* type V (PVL-positive) were identified. A correlation between MLST, *spa* and *agr* typing data was only achieved through *Apa*I MRA for PFGE pulsotype THW-V. Regarding PFGE pulsotypes THW-C, THW-J, and THW-O, it was seen that a corresponding MSSA strain was present for an MRSA strain with the same *spa* type, which might suggest local SCC*mec* acquisition. PFGE was identified as the most discriminatory technique, and PFGE/80% clustering as the most discriminatory clustering technique (Table 2). See Table 3 for all statistical associations identified.

Discussion

A prominent finding of this study is the high prevalence of *lukS/F* identified in 155 (42.2%) MSSA isolates. This is not in agreement with other international findings, which suggest that the prevalence of PVL is 3–5% [1]. Such a high PVL prevalence rate has previously been reported in Cambodia/Thailand [20], where PVL was associated with a better clinical outcome, as well as in sub-Saharan African countries [21]. Therefore, it appears that there is a significant difference in the PVL prevalence rate between developed and developing countries.

The PVL prevalence rate among MRSA isolates in this study was 9%, which was lower than in MSSA isolates. This may suggest that the PVL-positive MRSA isolates have acquired the phages encoding this virulence factor before acquiring SCC*mec* elements encoding methicillin resistance determinants. In this study, a large proportion of this *S. aureus* population consists of PVL-positive MSSA isolates from STs that are also associated with MRSA isolates. These isolates could thus conceivably be a source of new MRSA clones upon the acquisition of an SCC*mec* element. It is also worth noting that all PFGE complexes associated with skin and soft tissue infections (SSTIs) are also associated with PVL-positive isolates, which indicates either a strong linkage with crucial virulence factors for SSTIs or a pathogenetic role of PVL during the pathogenesis of these infections, as suggested by Lina *et al.* [18].

PFGE was an excellent technique for establishing clonal relatedness and dominance in our setting, and was also the most discriminatory method (Table 2). We used the PFGE results together with data generated through *spa*, *agr* and SCC*mec* typing to elucidate the population structure of *S. aureus*. PFGE identified 34 pulsotypes (nine

TABLE 2. The discriminatory power of the typing techniques used in this study

Method	No. of strains included	No. of types/ groups	DI	95% CI
spa typing	366[a]	127	0.965	0.956–0.974
spa typing/BURP	358[b]	16	0.890	0.877–0.902
PFGE[c]	366[a]	269	0.991	0.988–0.994
PFGE/80% clustering[c]	366[a]	31	0.918	0.905–0.931

DI, discriminatory index; BURP, Based Upon Repeat Pattern; PFGE, pulsed-field gel electrophoresis.
[a]One isolate was non-typeable.
[b]Types excluded from cluster analyses because they were too short.
[c]*Sma*I PFGE.

TABLE 3. Adjusted p-values for testing associations between clonality and the clinical/bacterial characteristics

			Bacterial/clinical characteristic				
PFGE	MLST (ST:CC)	Status of pulsotype	Methicillin resistance	Paediatric or adult	Source of infection	Gender	PVL
THW-A	1865:30	Major	**0.0096** (S)	1	**0.0096** (SSTI)	1	**0.0096** (+)
THW-B	36:30	Intermediate	**0.0096** (R)	0.055	0.35	1	**0.0096** (−)
THW-C	22:22	Major	**0.0096** (S)	1	**0.0096** (SSTI)	1	**0.0096** (+)
THW-E	121:121	Major	**0.0096** (S)	1	**0.0096** (SSTI)	1	**0.0096** (+)
THW-G	1862:8	Intermediate	**0.0096** (S)	0.055	0.35	1	1
THW-J	8:8	Intermediate	0.265	0.055	1	1	**0.0096** (−)
THW-L	239:8	Intermediate	**0.0096** (R)	1	1	1	**0.0096** (−)
THW-N	612:8	Intermediate	**0.0096** (R)	1	1	1	**0.0096** (−)
THW-O	612:8	Major	**0.0096** (R)	1	1	1	**0.0096** (−)
THW-S	15:15	Major	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-V	Various	Major	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-W	97:97	Intermediate	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-X	188:15	Intermediate	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-Y	1863:5	Major	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-Z	1864:5	Intermediate	**0.0096** (S)	1	1	1	**0.0096** (−)
THW-AA	5:5	Intermediate	**0.0096** (R)	1	1	1	**0.0096** (−)
THW-BB	88:88	Intermediate	0.26	1	0.35	1	**0.0096** (+)
THW-EE	45:45	Major	**0.0096** (S)	1	1	1	**0.0096** (−)

CC, clonal complex; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; PVL, Panton–Valentine leukocidin; R, resistant; S, sensitive; SSTI, skin and soft tissue; ST, sequence type; +, positive; −, negative.
Significant adjusted p-values (<0.05) are indicated in bold.

major, 11 intermediate, and 14 minor), whereas *spa* typing identified 16 *spa*-CCs by use of the BURP algorithm. The nine major and 11 intermediate PFGE pulsotypes yielded 19 MLST STs, which clustered into four CCs and seven singletons. It is worth noting that three MLST STs (ST1, ST6, and ST12) were identified that could not be discriminated with *Sma*I MRA. However, these were effectively discriminated with *Apa*I restriction enzyme. Isolates of the ST239-MRSA-III-t037 (CC8) background and of the CC30 background were clustered in a single *spa*-CC (CC021). This is because of the integration of a large chromosomal fragment from ST30 in ST8, leading to the evolution of ST239 [22]. As in previous studies, PFGE supplemented with *spa* typing and MLST could be used successfully in this study to investigate the population structure of *S. aureus* [19].

ST22-THW-C (major) was identified as the dominant MSSA clone. The presence of an MRSA isolate indicates that an SCC*mec* acquisition event may have occurred not too long ago, and this MRSA clone could be the first of a CA-MRSA lineage potentially associated with SSTI. ST22-MRSA-IV is usually associated with the epidemic MRSA clone EMRSA-15, which was a dominant clone in the UK for decades [23]. However, in our population it was established as the dominant MSSA clone, with one MRSA isolate with an SCC*mec* V element. The data thus suggest that this lineage may give rise to a CA-MRSA clone (possibly ST22-MRSA-V). ST8, ST612, ST239 and ST1862 were all identified as part of CC8. Two MRSA STs were identified. ST612, a double-locus variant of ST8, was the only ST that was subdivided by PFGE into two pulsotypes, THW-O (major) and THW-N (intermediate). One MSSA isolate was present in THW-O and had the same *spa* type as an MRSA isolate, t1443. It is speculated that this could possibly represent the local acquisition of SCC*mec* by MSSA isolates, leading to the rise of a local MRSA clone. As only one MSSA isolate was identified, it can be seen that the MSSA strains of this CC have been largely replaced by their MRSA counterparts. All members of ST8 carried *spa* t1476, and two MRSA isolates were also present, carrying SCC*mec* type V. These data, again, support the local acquisition of SCC*mec* elements, and these ST8-MRSA-V isolates might be the first of a new CA-MRSA clone. ST15 is known worldwide to be associated with MSSA isolates. Today, MRSA isolates from ST15 are scarce [24], although 'early' MRSA isolates have been identified from this ST [25]. ST1-MRSA-IV is associated with the USA400 clone [10,24], a CA-MRSA clone that is prevalent in the USA [10] (Table 3).

ST1863 and ST1864 were identified as novel MLST STs during this study, and they are both single-locus variants of ST5. Two MSSA lineages exist that are closely related to ST5 and are endemic to our setting. It is possible that this ST5 emerged locally after it underwent evolutionary changes after acquisition of an SCC*mec* I element.

ST121-MSSA-THW-E (major) is commonly only associated with an MSSA background, and has previously been reported [23,26]. This ST is a common, global cause of SSTIs [27]. Although MRSA isolates have been described from this ST, they are usually rare. Overall, our *S. aureus* population is very heterogeneous, especially the MSSA strains, and it is noted that there is a clear lack of a common genetic background between MSSA and MRSA strains. This either suggests that only some MSSA lineages come into contact with SCC*mec* elements or other bacterial species that can act as possible donors, that only some MSSA lineages can successfully sustain the integration of SCC*mec* elements in the genome, or that certain MRSA clones have lost the SCC*mec* element.

Two clones were identified as being associated with HIV-positive status: ST8-MSSA-THW-K (minor) and ST612-MRSA-IV-THW-P (minor). Both clones were associated with MLST CC8, which has been reported previously [28]. ST8 isolates have also been reported as USA300 isolates, and have been associated with necrotizing disease in HIV-positive persons [28]. ST612 has been described as the dominant ST, although from a different PFGE pulsotype. Further studies on the association of bacterial clonality with HIV-positive status are required.

Conclusion

The molecular typing techniques used in combination were sufficient to elucidate and study the population structure of *S. aureus*, allowing us to identify various clones, some of which were associated with certain clinical categories. The population studied is composed of a highly diverse population of endemic MSSA clones and epidemic MRSA clones, indicating the lack of a common genetic background between MSSA and MRSA isolates. A prominent finding is the high PVL prevalence, especially among MSSA clones. The MSSA population identified and studied could act as a potential reservoir for CA-MRSA clones upon the acquisition of SCC*mec* elements, leading to the rise of PVL-positive CA-MRSA clones. More studies are required to investigate the significant difference in the PVL prevalence rate between developed and developing countries. Some of the MRSA clones identified could potentially have emerged locally through the acquisition of SCC*mec* elements, including the unique, dominant MRSA clone, ST612.

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Author Contributions

The authors contributed as follows. W. Oosthuysen: designed and set up the study, collected *S. aureus* isolates, performed all experimental work, analysed and interpreted all data, and wrote the manuscript. H. Orth: supervised experimental work and reviewed the manuscript. C. Lombard: performed statistical analyses and reviewed the manuscript. B. Sinha: supervised experimental work and reviewed the manuscript. E. Wasserman: supervised experimental work and reviewed the manuscript.

Transparency Declaration

The authors declare no conflict of interest.

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